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Antifilarial activity of *Caesalpinia bonducella* against experimental filarial infections

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***Background & objectives*: Lymphatic filariasis is a disabling disease that continues to cripple population in tropical countries. Currently available antifilarial drugs are not able to control the disease. Therefore, a better antifilarial is urgently required for proper management of the disease. We undertook this study to assess the antifilarial activity of *Caesalpinia bonducella*-seed kernel against rodent filarial parasite in experimental model.**

***Methods*: Microfilaraemic cotton rats and *Mastomys coucha* harbouring *Litomosoides sigmodontis* and *Brugia malayi* respectively, were treated with crude extract or fractions of the seed kernel *C. bonducella* through oral route for 5 consecutive days. Microfilaricidal, macrofilaricidal and female worm sterilizing efficacy was assessed.**

***Results*: Crude extract showed gradual fall in microfilariae (mf) count in *L. sigmodontis*-cotton rat model from day 8 post-treatment attaining more than 95 per cent fall by the end of observation period. It also exhibited 96 per cent macrofilaricidal and 100 per cent female sterilizing efficacy. The butanol fraction F018 caused 73.7 per cent reduction in mf count and 82.5 per cent mortality in adult worms with 100 per cent female sterilization. The aqueous fraction F019 exerted more than 90 per cent microfilaricidal activity and 100 per cent worm sterilization. Two chromatographic fractions, F024 and F025 of hexane soluble fraction exhibited 64 and 95 per cent macrofilaricidal activity, respectively. Both the fractions caused gradual fall in microfilaraemia and 100 per cent worm sterilization. In *B. malayi-M. coucha* model F025 showed gradual reduction in microfilaraemia and caused 80 per cent sterilization of female parasites.**

***Interpretation & conclusions*: In conclusion, *C. bonducella*- seed kernel extract and fractions showed microfilaricidal, macrofilaricidal and female-sterilizing efficacy against *L. sigmodontis* and microfilaricidal and female-sterilizing efficacy against *B. malayi* in animal models, indicating the potential of this plant in providing a lead for new antifilarial drug development.**

Key words Antifilarial activity - *Brugia malayi* - *Caesalpinia bonducella* - *Litomosoides sigmodontis*

Lymphatic filariasis, a major vector-borne disease caused by the nematode parasites *Wuchereria bancrofti*, *Brugia malayi* and *B. timori*, causes chronic disability in the tropical and subtropical countries. In India, more than 553.7 million people are at risk[1] with 45.5 million people showing circulating microfilariae (mf) and another 22.5 million people suffering from chronic manifestations of the disease like hydrocele,

lymphoedema and elephantiasis[2]. Despite global efforts towards elimination of the disease by treatment with existing drugs and their combinations, the disease continues to spread.

The currently used drugs of choice diethylcarbamazine (DEC) and ivermectin are principally microfilaricides and have little activity against the adult worms. There is thus an urgent need for an agent that kills and/or sterilizes the adult worms, since adult parasites not only produce millions of mf that are picked up by mosquito vector and transmitted, but are also responsible for the debilitating pathological lesions. An ideal agent would be one that acts both on mf and macrofilariae (adult worms). Several medicinal agents have already been derived and developed from plants and utilized in traditional therapeutics. Although several plants have been used to develop anthelminthics, very few are specifically used to treat lymphatic filariasis. There are claims of antifilarial activity for *Azadirachta* indica[3] and *Pongamia pinnata*[4] against cattle filarial parasite *Setaria cervi, Xylocarpus granatum, Tinospora crispa* and *Andrographis paniculata* against *B. malayi*[5] and *Cardiospermum halicacabum* and *Neurolaena lobata* against *B. pahangi*[6,7]. Similarly *Centratherum anthelminticum, Cedrus deodara, Sphaeranthus indicus* and *Ricinus communis* were claimed to possess activity against *S. digiata*[8]. However, most of the claims were based on either *in vitro* studies or using filarial species that do not infect humans. Some promise has been shown by extracts/products of some plants of which the stem bark of *Streblus asper* was used clinically under the name "filacid" to treat lymhoedema, chyluria and other condition of the filarial disease but no claims were made of any direct effect on either mf or adult worms[9,10]. A systematic and detailed investigation on the antifilarial efficacy of the stem bark carried out by us showed macrofilaricidal/ adult sterilizing activity. The activity was localized in two cardiac glycosides, asperoside and strebloside[11]. Unfortunately, these glycosides are toxic due to their cardiotonicity.

Caesalpinia bonducella is a plant widely distributed throughout the warm regions of India and several preparations of the plant were used in folk medicine[12]. The seed of the plant is known as fever nut, bonduc nut and physic nut and the medicinal properties were mainly localized to the seed coat or seed kernel. There are claims that its leaves or seeds/seed kernel possess antipyretic, antidiuretic, antibacterial antiviral, anti-estrogenic and antidiabetic[13-20] activities. Although anthelmintic activity was also claimed for seed preparations of this plant[13], whether they are useful in treating lymphatic filariasis is not known. Phytochemical analysis of the seeds revealed the presence of flavonoids, terpenoids, glycosides, saponins, tanins and alkaloids[21-23].

We carried out this study to evaluate antifilarial activity of crude extract of seed kernel of *C. bonducella* and its various fractions against *Litomosoides carinii* (rodent filarial parasite) in cotton rats and target human filarial parasite *B. malayi* in *Mastomys coucha*.

Material & Methods

Collection of plant materials: Seeds of *C. bonducella* were collected from Lucknow and adjacent areas, and authenticated by Botany Division, Central Drug Research Institute (CDRI), Lucknow, India, and the herbarium (with voucher specimen # 6216) is preserved in the Division.

Extraction and fractionation: The air dried seed kernel was extracted exhaustively with 95 per cent ethanol to give the ethanolic extract, which was concentrated under reduced pressure below 45 ˚C using a rotavapor. The dried ethanolic extract was then treated with n-hexane and this gave an oil rich fraction F016 and residue. Fractionation of the residue with different solvents yielded three fractions: F017 (chloroform), F018 (butanol) and F019 (aqueous). Chromatography of oil rich F016 yielded 7 fractions (F020 to F026).

Animals: Cotton rats (*Sigmodon hispidus*) and *Mastomys coucha* used in the study were from our Institute's National Laboratory Animal Centre (NLAC). The animals were housed under controlled conditions of temperature (23 ±2 °C), humidity (RH: 60%) and photoperiod (12:12h light/dark cycle). They were fed on standard rodent chow and drinking water *ad libitum*. For *M. coucha*, rodent chow was supplemented with dried shrimps. The animals were sacrificed under deep ether anesthesia.The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC).

Host-parasite models: Two filarial species *L. sigmodontis* (animal filariid previously known as *L. carinii*) and *B. malayi* (subperiodic strain of human lymphatic dwelling filariid) maintained in rodents were used to evaluate antifilarial activity.

L. sigmodontis in cotton rat: Cotton rats (*S. hispidus*) were infected through exposure of infected mites *Liponyssus bacoti*[24, 25]. Mites were earlier fed on donor

cotton rats showing >1000 mf / 5 µl of blood. After the first appearance (60 days post-larval exposure) of mf in the peripheral blood of infected animals, blood examination was carried out weekly to assess the progressive increase in microfilaraemia. Animals showing progressive rise in microfilaraemia (from a starting mf load of 200-500 mf/5 µl blood) were used for the study.

B. malayi in *M. coucha*: Transmission of infection was carried out in animals broadly following the method described earlier[26-28]. Briefly, the infection in male *M. coucha* (6-8 wk old) was produced by inoculating infective larvae (L_3) obtained from experimentally infected laboratory-bred black eyed susceptible strain of *Aedes aegypti* mosquitoes which were fed 9-10 days before on microfilaraemic 100-200 mf/10 µl blood of *M. coucha*. Each animal received 100 L_3 (standard inoculum) through subcutaneous route.

Animals harbouring about 5-8 months old infection and showing a progressive increase in mf counts (starting mf load: 50-150 mf/10 µl blood) were selected for the study. Four to six animals in two experiments were included for each fraction or crude extract.

Administration of test materials and standard drug: Both crude extracts and fractions were suspended/ dissolved in 1 per cent gum acacia in triple distilled water[29]. Crude alcoholic extract of seed kernel of *C. bonducella* was tested at 1, 2 g/kg against *L. carinii* and 2 and 4 g/kg against *B. malayi*. Fractions were administered at 1 g/kg. The treatment was given through oral route for 5 consecutive days.

The dose selection is based on exploratory dose-range finding studies routinely followed in our laboratory. In this a starting dose of 1g/kg (oral) for extracts and 500 mg/kg (oral) for fractions are used. The dose is increased till acceptable activity is obtained. In the present case for the extract, we found a dose of 2g/kg in *L. sigmodontis* infection model and 4 g/kg in *B. malayi* model giving acceptable activity. Therefore the final doses for extracts were: 2 g/kg for *L. sigmodontis* and 4 g/kg for *B. malayi*. These doses were well tolerated by the animals and there was no mortality in the animals during the treatment and observation periods (42 days in *L. sigmodontis* infection; 90 days in *B. malayi* infection).

DEC citrate was used as the control drug for comparison under similar conditions against the infections. This drug was used at a dose of 12mg/kg (=6mg base) p.o. in case of *L. carinii*[11] and 100mg/kg (=50 mg base) i.p. in case of *B. malayi*[11,30]. Both test materials and standard drug were given for 5 consecutive days.

Sex-, age- and infection-matched untreated animals receiving vehicle only were kept as controls.

Assessment of antifilarial activity: Micro- and macrofilaricidal efficacy of the plant products was assessed according to the methods of Lammler *et al*[31]. Five µl of tail blood of treated and control animals were examined just before treatment and thereafter at weekly intervals until completion of observation period of 42 (in case of *L. carinii*) and 91 days (in case of *B. malayi*). The intensity of microfilaricidal activity as per cent change in population of mf over pretreatment levels was calculated.

Macrofilaricidal activity was assessed following the method of Chatterjee *et al*[11]. Treated as well as control animals were sacrificed on day 42 or 91 post-treatment. Adult males and females were isolated from pleural cavity (in case of cotton rat) and various tissues (in case of *M. coucha*) of the animals and examined in normal saline for motility and cell adhesion on the surface of the worm. The per cent mortality of adult filariids was calculated by comparing the live worms recovered from treated animals with that of total number of living worms recovered from untreated (control) animals. All surviving females were teased individually in saline on glass slides and the conditions of mf and its developing forms *in uteri* were examined under microscope. Female worms with empty uteri or uteri containing dead or degenerated embryos or mf were considered sterile.

Statistical analysis: Results were presented as mean ± SD of 4-6 animals in two independent experiments and the data were analyzed statistically using GraphPad Prism®.

Results

The animals tolerated the test substances well at the doses employed and there was no mortality during the treatment and observation periods (42 days in *L. sigmodontis* infection; 90 days in *B. malayi* infection).

Against L. sigmodontis in cotton rats: At 2 g/kg, p.o. x 5 days, the alcoholic extract of seed kernel showed 60.7, 72.4 and 98.4 per cent microfilaricidal action respectively on day 8, 21 and 42 post-treatment with 96.0 per cent adulticidal activity. All the surviving females contained dead and degenerated uterine content. Fractions were used at 1g/kg p.o. x 5 days. The oil rich hexane fraction (F016) showed gradual reduction in microfilaraemia from day 8 to day 42 but with little less macrofilaricidal

(77.5%) action. F017 (chloroform), was devoid of antifilarial activity. Butanol fraction (F018) caused 73.7, 86.7 and 100 per cent fall in mf count respectively, on days 8, 21 and 42 and 82.5 per cent dead adult worms with 100 per cent female sterilization. Aqueous fraction (F019) showed more than 90 per cent microfilaricidal action on day 8 and by day 42 the animals were completely free from mf. On autopsy, 100 per cent of female worms were sterile (Table I).

Of the seven fractions (F020 - F026) two fractions (F024 and F025) exhibited significant antifilarial activity. Both the fractions exhibited almost similar pattern of microfilaricidal activity. However, F025 showed 96 per cent macrofilaricidal activity with 100 per cent sterilizing effect on female reproductive potential (Table I).

Treatment with DEC (12mg/kg, p.o. x 5 days) caused a sharp fall in microfilaraemia on day 8 since the start of the treatment followed by gradual reappearance of microfilaraemia by end of the observation period (day 42). Adult worms were not affected. Untreated control animals showed progressive increase in mf count with live and motile worms (Table I).

Against B. malayi in M. coucha: Alcoholic extract (crude) of seed kernel of *C. bonducella* at 4g/kg, p.o x 5 days did not exert any effect against mf on day 8 post-treatment but it killed around 32 per cent adult worms (Table II). Only one (F025) out of seven fractions (1g/kg, p.o x 5 days) exhibited gradual fall in microfilaraemia with maximum effect (63%) on day 91. No significant per cent death of adult worms occurred but a significant number of female worms (around 80%) revealed dead and degenerated uterine contents. DEC at 100 mg/kg given through intraperitoneal route showed 95.2 and 91.6 per cent microfilaricidal action, respectively, on days 8 and 91 (Table II).

Discussion

In the present study crude extract of *C. bonducella* at 2g/kg killed more than 90 per cent of adult parasites

Table I. Antifilarial activity of *C. bonducella* against *Litomosoides carinii* in cotton rats

Crude extract/ fractions	Dose (mg/kg), p.o. x 5 days (n)	Per cent reduction in Mf count (day post treatment)			Per cent mortality in adult worms over control	% Sterilization of surviving females over control
		8	21	42	Total	
Crude (Alcoholic)	2000 (6)	60.7±24.3	72.4±18.4	98.4±3.0	96.0±4.6**	100
F016	1000 (5)	28.4±9.7	86.1±19.5	92.3±10.8	77.5±3.5**	100
F018	1000 (4)	73.7±10.8	86.7±18.7	100	82.5±24.7**	100
F019	1000 (4)	93.2±9.6	86.1±18.7	100	0	100
F024	1000 (4)	43.6±14.1	41.8±26.4	92.5±2.8	64.4±3.1**	100
F025	1000 (4)	59.7±1.5	37.1±27.0	98.5±2.0	95.5±6.2**	100
DEC	12 (5)	94.4±4.5	0	0	0	1.5±0.5
Control (untreated)	Vehicle (5)	0	0	0	#	$

n, No. of animals; #Adult worm recovery from control= 20.0±4.2; $Sterile female=0; **$P<0.001$ (values are mean ±SD)

Table II. Antifilarial activity of *C. bonducella* against *Brugia malayi* in *Mastomys coucha*

Crude extract/ fractions	Dose (mg/kg), p.o. x 5 days (n)	Per cent reduction in Mf count (day post treatment)				Per cent mortality in adult worms over control	% Sterilization of surviving females over control
		8	21	42	91	Total	
Crude (alcoholic)	4000 (6)	0	-	0	0	26.1±16.0	32.1±5.9*
Control	Vehicle (4)	0	0	0	0	@	@@
F025	1000 (4)	19.5±40.0	-	55.5±15.5	63.3±9.5	29.6±35.7	80.11±11.6**
DEC	100 i.p. (5)	95.92±4.6		71.6±13.5	91.6±9.3	53.3±26.7	0
Control (untreated)	Vehicle (4)	0	0	0	0	#	##

n, No. of animals; @Adult worm recovery from control (for crude extract) = 23.7±10.2; @@11.5±1.8; #Adult worm recovery from control kept (for F025) = 18.0±6.4; ##13.2±7.9; *$P< 0.05$; **$P< 0.001$ (values are mean ± SD)

of *L. sigmodontis.* As the extract contains lots of oil, it was treated with n-hexane to separate the oily portion. The chemical examination of seeds of *C. bonducella* as carried out by Khuda and Irfan[32] revealed that the kernel portion contained oil, saponin, starch, sucrose and two other compounds, α- and β-caesalpin. Purushothaman *et al*[22] isolated one more caesalpin from the seeds. In the present study, oily portion (F016) showed significant adulticidal activity. Although butanol fraction F018 also showed activity comparable to F016, the yield of the former was very poor. Chromatographic fractions F024 and F025 revealed good antifilarial activity in *L. sigmodontis* system; of these F025 was the most active one with respect to adulticidal and female sterilizing activity. Fraction F025 was also active against *B. malayi* in *M. coucha* where it produced a gradual decrease in mf counts with maximum reduction occurring at the end of the observation period and a significant sterilization effect on female worms. The crude extract killed more than 30 per cent of *B. malayi* adult worms. Interestingly, despite the potent adulticidal action, both the crude extract and the fractions were well tolerated by the animals at the doses employed and there was no mortality during the treatment and observation periods. Comprehensive regulatory toxicity studies need to be done.

In conclusion, *C. bonducella*- seed kernel showed microfilaricidal, macrofilaricidal and female-sterilizing efficacy against *L. sigmodontis* and microfilaricidal and female-sterilizing efficacy against *B. malayi* in animal models, indicating the potential of this plant in providing a lead for new antifilarial drug development.

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